

Sprouts of the broccoli cultivar Everest contained 130-fold more inducer potential (units/g fresh weight) than mature vegetables. The inducer activity in broccoli was significantly higher than in daikon.

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*Example 5**INDUCER POTENTIAL OF BROCCOLI SPROUT EXTRACTS*

Inducer potential of a series of water extracts of 3-day old broccoli sprouts of the cultivar Saga were determined. Plants were prepared by first surface  
 10 sterilizing seeds of *Brassica oleracea* variety *italica* (broccoli) cultivar Saga by a 1 min treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite containing approximately 0.001% Alconox detergent. Seeds  
 15 were grown in sterile plastic containers at a density of approximately 8 seeds/cm<sup>2</sup> for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C).

20 Plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. Sprouts (approximately 25 mg fresh wt/sprout) were gently harvested and immediately and rapidly plunged into  
 25 approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase as well as to extract glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for 3 min. The sprouts were then either strained  
 30 from the boiled infusion [tea, soup] or homogenized in it, and the residue then removed by filtration or centrifugation.

Data in Table 3 represent both homogenates and infusions. Preparations were stored at -20°C until  
 35 assayed. Inducer potential of plant extracts, prepared

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as described above, was determined as described in  
Definitions section above.

TABLE 3  
Inducer Potentials of Hot Water Extracts  
of 3-Day Saga Broccoli Sprouts

EXTRACT NO.	units/g fresh weight
1	500,000
2	370,000
3	455,000
4	333,000
5	435,000
6	333,000
7	625,000
8	250,000
9	313,000
10	357,000
11	370,000
12	370,000
13	217,000
14	222,000
15	1,000,000
16	714,000
17	435,000
18	1,250,000
19	263,000
AVERAGE	464,000 $\pm$ 61,600 S.E.M.

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Some variability in the amount of Phase 2 enzyme-inducer potential was detected. High levels of Phase 2 enzyme-inducer potential, however, were consistently observed.

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**Example 6**

**HOT WATER BROCCOLI EXTRACTS TREATED  
WITH DAIKON MYROSINASE**

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QR activity in a hot water broccoli extract increased in the presence of a vegetable source of myrosinase. An aqueous extraction of 3-day old sprouts of broccoli cultivar Saga grown on water agar, in which myrosinase was inactivated by boiling for 3 min, was divided into 6 different 150 ml aliquots. Nine-day old daikon sprouts, a rich source of the enzyme myrosinase, were added to this cooled infusion in amounts equivalent to 0, 5, 9, 17, 29 and 40% (w/w) of the broccoli. QR activity, as determined in the Definition section, of the control extracts containing 0% daikon was 26,300 units/gram fresh weight while QR activity of the extracts that had received daikon as a source of myrosinase ranged from 500,000 to 833,000 units/gram fresh weight of broccoli. Accordingly, myrosinase present in the daikon sprouts, increased the QR activity in the broccoli extract greater than 19-fold.

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**Example 7**

**GLUCORAPHANIN AND GLUCOERUCIN ARE THE PREDOMINANT  
GLUCOSINOLATES IN HOT WATER EXTRACTS OF BROCCOLI  
(CULTIVAR SAGA) SPROUTS**

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Paired Ion Chromatography (PIC). Centrifuged hot water extracts of 3-day-old broccoli (cultivar Saga) sprouts were subjected to analytical and preparative PIC on a reverse phase C18 Partisil ODS-2 HPLC column in ACN/H<sub>2</sub>O (1/1, by vol.) with tetraoctylammonium (TOA) bromide as the counter-ion. Only three well-separated peaks were detected: peak A eluted at 5.5 min, B at 11.5

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min, and C at 13 min at a molar ratio [A:B:C] of ca. 2.5 : 1.6 : 1.0 (monitored by UV absorption at 235 nm), and they disappeared if the initial extracts were first treated with highly purified myrosinase. Peaks A, B, and C contained no significant inducer activity, and cyclocondensation assay of myrosinase hydrolysates showed that only Peaks A and C produced significant quantities of isothiocyanates, accounting for all the inducer activity. See Zhang et al., *Anal. Biochem.* 205: 100-107 (1992). Peak B was not further characterized. Peaks A and C were eluted from HPLC as TOA salts but required conversion to ammonium salts for successful mass spectroscopy, NMR and bioassay. The pure peak materials were dried in a vacuum centrifuge, redissolved in aqueous 20 mM  $\text{NH}_4\text{Cl}$ , and extracted with chloroform to remove excess TOA bromide. The ammonium salts of glucosinolates remained in the aqueous phase, which was then evaporated.

*Identification of Glucosinolates.* The ammonium salts of Peaks A and C were characterized by mass spectrometric and NMR techniques: (a) negative ion Fast Atom Bombardment (FAB) on a thioglycerol matrix; this gave values of 436 (Peak A) and 420 (Peak C) amu for the negative molecular ions, and (b) high resolution NMR, as shown in Figure 2, provided unequivocal identification of the structure. Peak A is glucoraphanin [4-methylsulfinylbutyl glucosinolate], and Peak C is the closely related glucoerucin [4-methylthiobutyl glucosinolate]. These identifications and purity are also consistent with the inducer potencies; Peaks A and C, after myrosinase hydrolysis had potencies of 36,100 and 4,360 units/ $\mu\text{mol}$ , respectively, compared with reported CD values of 0.2  $\mu\text{M}$  (33,333 units/ $\mu\text{mol}$ ) for sulforaphane and 2.3  $\mu\text{M}$  (2,900 units/ $\mu\text{mol}$ ) for erucin. CD values are the concentrations of a compound required to double the QR specific activity in Hepa 1c1c7 murine hepatoma cells. Since there are no other glucosinolate peaks, and the inducer activity of peak A and C account for the total inducer activity of the extracts, it is

therefore likely that in this cultivar of broccoli, there are no significant quantities of other inducers, i.e., no indole or hydroxyalkenyl glucosinolates. Further, the isolated compounds are therefore substantially pure.

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**Example 8****COMPARISON OF AQUEOUS AND ORGANIC SOLVENT TECHNIQUES  
FOR EXTRACTION OF INDUCER POTENTIAL**

Plants were prepared by first surface sterilizing seeds of *Brassica oleracea* variety *italica* (broccoli) cultivar Saga, with 70% ethanol followed by 1.3% sodium hypochlorite and 0.001%alconox. The seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm<sup>2</sup> for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity, and temperature control (16 hours light, 25°C/8 hours dark, 20°C).

The plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. A portion of the plants was homogenized with 10 volumes of the DMF/ACN/DMSO solvent at -50°C, as described in Example 1, which dissolves nearly all the non-lignocellulosic plant material. Alternatively, the bulk of the harvested plants was plunged into 5 volumes of boiling water for 3 min to inactivate endogenous myrosinase and to extract glucosinolates and isothiocyanates. The cooled mixture was homogenized, centrifuged, and the supernant fluid was stored at -20°C.

Inducer potential of plant extracts, prepared by the two methods described above, was determined by the microtiter plate bioassay as described above. Typical inducer potentials in an average of 5 preparations were 702,000 (DMF/ACN/DMSO extracts) and 505,000 (aqueous extracts) units/g fresh weight of sprouts.

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Spectrophotometric quantitation of the cyclocondensation product of the reaction of isothiocyanates with 1,2-benzenedithiole was carried out as described in Zhang et al., *Anal. Biochem.* 205: 100-107 (1992). Glucosinolates were rapidly converted to isothiocyanates after addition of myrosinase. About 6% of the total hot water extractable material [dissolved solids] consisted of glucosinolates. These results demonstrate that (a) isothiocyanate levels in the crude plant extracts are extremely low; (b) myrosinase rapidly converts abundant glucosinolates to isothiocyanates; (c) hot water extraction releases over 70% of the inducer activity extractable with a triple solvent mixture permitting recovery of most of the biological activity in a preparation that is safe for human consumption; and (d) over 95% of the inducing potential in the intact plant is present as glucosinolates and therefore no other inducers are present in biologically significant quantities.

#### Example 9

#### 20 DEVELOPMENTAL REGULATION OF GLUCOSINOLATE PRODUCTION

Preliminary experiments in which field grown broccoli (cultivar DeCicco) was harvested at sequential time points from the same field indicated that on a fresh weight basis, inducer potential declined from the early vegetative stage through commercial harvest, but appeared to increase at late harvest (onset of flowering). These data suggested that inducer potential might be highest in seeds. Subsequent studies have shown that when seeds of 8 broccoli cultivars were surface sterilized and grown under gnotobiotic conditions, Phase 2 enzyme-inducer potential was highest in seeds and declined progressively (on a fresh weight basis) over time throughout the first 14 days of seedling growth.

Expressed on a per plant basis, however, activity remained constant over this period, suggesting that at

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5 this early stage of growth there was no net synthesis of glucosinolates. However, when the glucosinolate profiles of market stage broccoli heads and 3 day old sprouts (cultivar Emperor) were compared, there was a profound difference in the apparent glucosinolate compositions of these plants.

10 Sprouts were prepared by first surface sterilizing seeds of *Brassica oleracea* variety *italica* (broccoli) cultivar Emperor with a 1 minute treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite with approximately 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm<sup>2</sup> for 72 hours on a 0.7% agar support that did not contain added nutrients. The  
15 environment was carefully controlled; broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C).

20 Plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. Sprouts [approximately 25 mg fresh wt/sprout], were gently harvested and immediately and rapidly plunged into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase as well as to extract  
25 glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for 3 min. The sprouts were then strained from the boiled infusion [tea, soup] and the infusion was stored at -20°C until assayed.

30 Market stage heads were obtained by germinating seeds of the same seedlot in a greenhouse in potting soil, transplanting to an organically managed field in Garrett County, MD and harvested at market stage. Heads were immediately frozen upon harvest, transported to the  
35 laboratory on ice and extracts were prepared in an identical fashion to those described above for sprouts

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except that approximately 3 gram floret tissue samples were used for extraction.

Inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described in Example 1. Paired ion chromatography revealed two major peaks, probably glucobrassicin and neo-glucobrassicin, in extracts of market stage heads with similar retention times to glucobrassicin (indole-3-ylmethyl glucosinolate) and neo-glucobrassicin (1-methoxyindole-3-ylmethyl glucosinolate). This observation is consistent with published reports on the glucosinolate composition of mature broccoli plants. However, paired ion chromatography under the same conditions of identically prepared extracts of 3-day-old sprouts showed absence of glucobrassicin or neo-glucobrassicin. Additionally, 3-day-old sprouts of different broccoli cultivars produce different mixtures of glucosinolates. Accordingly, glucosinolate production is developmentally regulated.

#### Example 10

#### EVALUATION OF ANTICARCINOGENIC ACTIVITIES OF BROCCOLI SPROUT PREPARATIONS IN THE HUGGINS DMBA (9,10 DIMETHYL-1,2-BENZANTHRACENE) MAMMARY TUMOR MODEL

Sprouts were prepared by first surface sterilizing seeds of *Brassica oleracea* variety *italica* (broccoli) cultivar Saga with a 1 min treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite with approximately 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm<sup>2</sup> for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C).



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The plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. A large quantity of sprouts was harvested by immediately  
 5 and rapidly plunging into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase, as well as extracting glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for  
 10 3 min. Sprouts were then strained from the boiled infusion [tea, soup] and the infusion was lyophilized and stored as a dry powder at -20°C [designated Prep A]. Other sprouts, similarly prepared were extracted with boiling water, cooled to 25°C and were amended with a  
 15 quantity of 7 day old daikon sprouts equivalent to approximately 0.5% of the original fresh weight of broccoli sprouts. This mixture was homogenized using a Brinkman Polytron Homogenizer and incubated at 37°C for 2 hours following which it was filtered through a  
 20 sintered glass filter, lyophilized as above and stored as a dried powder at -20°C [designated Prep B].

QR inducer activity and inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described above.  
 25 The induction of QR activity in preparation A is largely due to glucosinolates; predominantly glucoraphanin, which is the glucosinolate of sulforaphane, but this preparation also contains some glucoerucin, which is the sulfide analog of glucoraphanin. The induction QR  
 30 activity of preparation B is almost exclusively due to isothiocyanates arising from treatment of glucosinolates with myrosinase.

Female Sprague-Dawley rats received at 35 days of age were randomized; 4 animals per plastic cage. All animals  
 35 received 10 mg DMBA, by gavage in 1 ml sesame oil, at age 50 days. Sprout preparations (A or B) or vehicle control were given by gavage at 3, 2 & 1 day prior to DMBA, on

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the day of DMBA (2 hr prior to the DMBA dose) and on the  
day following DMBA dosing. The vehicle used was 50%  
Emulphor 620P / 50% water. Animals were maintained on a  
semi-purified AIN-76A diet *ad libitum* from the time of  
5 receipt until termination of the experiment (167 days of  
age).

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TABLE 4

ANTICARCINOGENIC ACTIVITIES OF BROCCOLI SPROUT EXTRACTS  
IN THE DMBA RAT MAMMARY TUMOR MODEL

GROUP	TREATMENT	NUMBER OF ANIMALS AT TERMINATION	TOTAL TUMOR NUMBER	MULTIPLICITY: NUMBER OF TUMORS PER RAT
CONTROL	DMBA only	19	34	1.79
PREPARATION A (Glucosinolate)	324 mg/dose (100 $\mu$ mol sulforaphane equiv.)	18	19	1.05
PREPARATION B (Isothiocyanate)	424 mg/dose (100 $\mu$ mol sulforaphane equiv.)	20	11	0.55

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The development of palpable tumors was delayed for as much as 5 weeks by the administration of sprout extracts. Rats treated with either Preparation A or B had significantly fewer tumors than the untreated control, and the multiplicity of tumors (tumors per rat) was significantly lower in the animals receiving Preparations A or B.

#### Example 11

#### **METABOLISM AND CLEARANCE OF GLUCOSINOLATES IN HUMANS**

Two male, non-smoking volunteers ages 35 and 40 years, each in good health, were put on a low vegetable diet in which no green or yellow vegetables, or condiments, mustard, horseradish, tomatoes or papayas were consumed. After 24 hours on such a diet, all urine was collected in 8 hr aliquots. After 24 hours of baseline data, subjects ingested 100 ml of broccoli sprout soup (prepared as below), containing 520  $\mu$ mol of glucosinolates.

The sprouts were prepared by first surface sterilizing seeds of *Brassica oleracea* variety *italica* (broccoli) cultivar Saga with a 1 min treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite with ca. 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm<sup>2</sup> for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C). The plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. A large quantity of sprouts was harvested by immediately and rapidly plunged into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase as

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well as to extract glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for 3 min. Following the boiling step, sprouts were homogenized directly in their infusion water for 1 min using a Brinkman Polytron Homogenizer and the preparations were frozen at -79°C until use.

Inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described above. Inducer potential is nearly all due to glucosinolates; predominantly glucoraphanin, which is the glucosinolate of sulforaphane, but some glucoerucin which is the sulfide analog of glucoraphanin was also present. When converted to isothiocyanates by the addition of purified myrosinase, Phase 2 enzyme-inducing potential was 100,000 units/ml and contained 5.2  $\mu\text{mol}$  of isothiocyanates per ml, as determined by the cyclocondensation reaction described in Example 7. Thus, the subjects consumed a total of 520  $\mu\text{mol}$  of glucosinolates.

Collection of 8 hour urine samples was continued for an additional 30 hours. Urinary excretion of isothiocyanate conjugates (dithiocarbamates) was monitored using the cyclocondensation reaction as described in Example 7.

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**TABLE 5**  
**EXCRETION OF DITHIOCARBAMATES BY TWO SUBJECTS**  
**INGESTING 520 MICROMOLES OF GLUCOSINOLATES**  
**EXTRACTED FROM SAGA BROCCOLI**

TIME	CONDITION	SUBJECT 1	SUBJECT 2
Collection Time (hours)		$\mu$ mol Dithiocarbamate per 8 hour urine collection	
8	baseline	1.4	2.7
16	baseline	2.1	0.9
24	baseline	1.7	5.4
32	1st 8 hour post-dose	23.2	20.4
40	2nd 8 hour post-dose	9.9	36.8
48	3rd 8 hour post-dose	4.4	14.0
56	4th 8 hour post-dose	4.2	4.1
Total post-dose minus average baseline:		39.8	63.2
Total as Percent of dose:		6.7%	12.2%

The two subjects studied metabolically converted a significant fraction of the ingested glucosinolates to the isothiocyanates which were converted to cognate dithiocarbamates and measured in the urine.

**Example 12**

**EFFECTS OF PHYSICAL INTERVENTIONS ON SPROUT GROWTH**  
**ON PRODUCTION OF INDUCERS OF QUINONE REDUCTASE**

Sprouts were prepared by first surface sterilizing seeds of *Raphanus sativum* (daikon) by a 1 minute treatment with 70% ethanol, followed by a 15 min treatment with 1.3% sodium hypochlorite with approximately 0.001% Alconox detergent. Seeds were grown

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in sterile plastic containers at a density of approximately 8 seeds/cm<sup>2</sup> for 7 days on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light 25°C/8 hours dark, 20°C).

Treated sprouts were irradiated with germicidal UV light for 0.5 hr on days 5 and 6. Treated sprouts were only half the height of the untreated controls. Plants were harvested on day 7 by rapidly and gently collecting the plants from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. Sprouts were harvested by immediate and rapid plunging into approximately 10 volumes of DMF/ACN/DMSO (1:1:1) at approximately -50°C in order to inactivate endogenous myrosinase as well as to extract glucosinolates and isothiocyanates. Sprouts were immediately homogenized with a ground glass mortar and pestle and stored at -20°C.

Inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described above. Inducer potential of the UV-treated sprouts was over three times that of untreated controls. Treatment of sprouts with ultraviolet light therefore increased the Phase 2 enzyme-inducer potential of the plant tissue.

Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention, which is defined by the following claims. All publications and patent applications mentioned in this specification are indicative of the

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level of skill of those in the art to which the invention  
pertains.

5 All publications and patent applications are herein  
incorporated by reference to the same extent as if each  
individual publication or patent application were  
specifically and individually indicated to be  
incorporated by reference in its entirety.

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*What Is Claimed Is:*

1. Cruciferous sprouts, with the exception of cabbage, cress, mustard and radish sprouts, harvested prior to the 2-leaf stage.

2. The cruciferous sprouts according to claim 1, wherein said sprouts are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*, *gemmifera*, *gongylodes*, *italica*, *medullosa*, *palmifolia*, *ramosa*, *sabauda*, *sabellica*, and *selensia*.

3. The cruciferous sprouts according to claim 2, wherein said sprouts are a *Brassica oleracea* variety *italica*.

4. The cruciferous sprouts according to claim 1, wherein said sprouts are a *Brassica oleracea* variety *botrytis*.

5. The cruciferous sprouts according to claim 1, wherein said sprouts are a *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.

6. The cruciferous sprouts according to claim 1, wherein said sprouts are substantially free of Phase 1 enzyme-inducing potential.

7. A non-toxic solvent extract of the cruciferous sprouts according to claim 1.

8. The non-toxic solvent extract according to claim 7, wherein said solvent is water.

9. The non-toxic solvent extract according to claim 8, further comprising a cruciferous vegetable comprising an active myrosinase enzyme.

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10. The non-toxic solvent extract according to claim 9, wherein said cruciferous vegetable is of the genus *Raphanus*.

11. A method of increasing the chemoprotective amount of Phase 2 enzymes in a mammal, comprising the step of administering an effective quantity of the cruciferous sprouts according to claim 1.

12. Cruciferous sprouts harvested prior to the 2-leaf stage, wherein said sprouts have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth from seeds that produce said sprouts and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates.

13. The cruciferous sprouts according to claim 12, wherein said sprouts are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*, *gemma*, *gongylodes*, *italica*, *medullosa*, *palmifolia*, *ramosa*, *sabauda*, *sabellica*, and *selensia*.

14. The cruciferous sprouts according to claim 13, wherein said sprouts are a *Brassica oleracea* variety *italica*.

15. The cruciferous sprouts according to claim 13, wherein said sprouts are a *Brassica oleracea* variety *botrytis*.

16. The cruciferous sprouts according to claim 15, wherein said sprouts are a *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.

17. A non-toxic solvent extract of the cruciferous sprouts according to claim 12.

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18. The non-toxic solvent extract according to claim 17, wherein said solvent is water.

19. The non-toxic solvent extract according to claim 18, further comprising a cruciferous vegetable comprising an active myrosinase enzyme.

20. The non-toxic solvent extract according to claim 19, wherein said cruciferous vegetable is of the genus *Raphanus*.

21. A method of preparing a food product rich in glucosinolates, comprising germinating cruciferous seeds, with the exception of cabbage, cress, mustard and radish seeds, and harvesting sprouts prior to the 2-leaf stage, to form a food product comprising a plurality of sprouts.

22. The method according to claim 21, wherein said sprouts contain non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates.

23. The method according to claim 21, wherein said seeds are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*, *gemnifera*, *gongylodes*, *italica*, *medullosa*, *palmifolia*, *ramosa*, *sabauda*, *sabellica*, and *selensia*.

24. The method according to claim 23, wherein said seeds are *Brassica oleracea* variety *italica*.

25. The method according to claim 23, wherein said seeds are *Brassica oleracea* variety *botrytis*.

26. The method according to claim 25, wherein said seeds are *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.

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27. A food product rich in glucosinolates made by the process according to claim 21.

28. A method of preparing a food product, comprising extracting glucosinolates and isothiocyanates from cruciferous sprouts according to claim 1 with a non-toxic solvent, removing the extracted sprouts from said solvent, and recovering the extracted glucosinolates and isothiocyanates.

29. A method of preparing a food product according to claim 28, wherein active myrosinase enzyme is mixed with said cruciferous sprouts, or said extracted glucosinolates and isothiocyanates, or both said cruciferous sprouts or said extract.

30. A method of preparing a food product rich in glucosinolates, comprising germinating cruciferous seeds that produce sprouts having at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth and which contain non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates, and harvesting sprouts prior to the 2-leaf stage to form a food product comprising a plurality of sprouts.

31. The method according to claim 30, wherein said seeds are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*, *gemmifera*, *gongylodes*, *italica*, *medullosa*, *palmifolia*, *ramosa*, *sabauda*, *sabellica*, and *selensia*.

32. The method according to claim 31, wherein said seeds are *Brassica oleracea* variety *italica*.

33. The method according to claim 31, wherein said seeds are *Brassica oleracea* variety *botrytis*.

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34. The method according to claim 33, wherein said seeds are *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.

35. A food product rich in glucosinolates, made by the process according to claim 30.

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36. A method of preparing a food product, comprising introducing cruciferous seeds, wherein said seeds produce sprouts having at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates, into another edible ingredient.

37. A method of preparing a food product, comprising extracting glucosinolates and isothiocyanates with a non-toxic solvent and isothiocyanates from cruciferous seeds, sprouts, plants or plant parts wherein seeds that produce said sprouts, plant, or plant parts, have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth and wherein said seeds, sprouts, plants or plant parts have non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates, and recovering the extracted glucosinolates and isothiocyanates.

38. A method of preparing a food product according to claim 37, wherein active myrosinase enzyme is mixed with said cruciferous seeds, sprouts or plants; or said extracted glucosinolates and isothiocyanates; or both said cruciferous seeds, sprouts or plants and said extract.

39. A method of reducing the level of carcinogens in a mammal, comprising administering to a mammal an

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effective amount of cruciferous sprouts, with the exception of cabbage, cress, mustard and radish sprouts.

40. A method of reducing the level of carcinogens in a mammal, comprising administering to a mammal an effective amount of cruciferous sprouts having at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth from seeds that produce said sprouts and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates.

41. A method of extracting glucosinolates and isothiocyanates from plant tissue comprising the steps of homogenizing said plant tissue in an excess of a mixture of dimethyl sulfoxide, acetonitrile and dimethylformamide at a temperature sufficient to inactivate myrosinase enzyme activity.

42. A food product comprising cruciferous sprouts, with the exception of cabbage, cress, mustard and radish sprouts, harvested prior to the 2-leaf stage, cruciferous seeds; extracts of said sprouts or seeds; or any combination of said sprouts, seeds or extracts.

43. A method of increasing the chemoprotective amount of Phase 2 enzymes in a mammal, comprising the step of administering an effective quantity of the food product according to claim 42.

44. A food product comprising cruciferous sprouts harvested prior to the 2-leaf stage, wherein said sprouts have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth from seeds that produce said sprouts and non-toxic levels of indole glucosinolate and goitrogenic hydroxybutenyl glucosinolates; cruciferous seeds; extracts of said sprouts or seeds; or any combination of said sprouts, seeds or extracts.

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45. A method of increasing the chemoprotective amount of Phase 2 enzymes in a mammal, comprising the step of administering an effective quantity of the food product according to claim 44.

46. Cruciferous sprouts harvested prior to the 2-leaf stage, wherein the ratio of monofunctional to bifunctional inducers is at least 20 to 1.

47. A food product supplemented with a purified or partially purified glucosinolate.

add B15  
add C3

665207-06852460

Docket No. 46528/102/JOHO**DECLARATION AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.  
 I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**CANCER CHEMOPROTECTIVE FOOD PRODUCTS**

the specification of which (check one)

☒ is attached hereto☐ was filed on \_\_\_\_\_ as Application Serial No. \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

**PRIOR FOREIGN APPLICATION(S)**

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; John J. Feldhaus, Reg. No. 28,822; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

Send all correspondence to **FOLEY & LARDNER**, 3000 K Street, N.W., Suite 500, Washington, DC 20007-5109. Address telephone communications to Bernhard D. Saxe at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor <b>Jed W. FAHEY</b>	Signature of First or Sole Inventor <i>Jed W. Fahey</i>	Date <b>9/13/95</b>
Residence Address <b>6704 RIDGE RD., ELDERSBURG, MD 21784</b>		Country of Citizenship <b>United States</b>
Post Office Address <b>6704 RIDGE RD., ELDERSBURG, MD 21784</b>		

Signatures should conform to names as typewritten. ☒ Additional inventors on attached Page 2.



PAGE 2

Docket No. 46528/102/JOHQ

Full Name of Second Inventor <i>Paul TALALAY</i>	Signature of Second Inventor <i>Paul Talalay</i>	Date <i>9/13/95</i>
Residence Address <i>5512 BOXHILL LANE, BALTIMORE MD 21210</i>		Country of Citizenship <i>United States</i>
Post Office Address <i>5512 BOXHILL LANE BALTIMORE MD 21210</i>		

09425890-102599  
665201

Applicant or Patentee: FAHL et al.  
 Serial or Patent No.: 08/528,858 Atty. Dkt. No. 46528/102/JOHO  
 Filed or Issued: 9/15/95  
 For: CANCER CHEMOPROTECTIVE FOOD PRODUCTS

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
 (37 CFR 1.9(f) AND 1.27 (c)) — NONPROFIT ORGANIZATION**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: Johns Hopkins School of Medicine  
 ADDRESS OF ORGANIZATION: 2024 E. Monument Street, Suite 2-100, Baltimore, MD 21205  
 TYPE OF ORGANIZATION:  
☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION  
☐ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) AND 501(c)(3))  
☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA  
 (NAME OF STATE )  
 (CITATION OF STATUTE )  
☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3) IF LOCATED IN THE UNITED STATES OF AMERICA  
☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA  
 (NAME OF STATE )  
 (CITATION OF STATUTE )

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under section 41(a) or (b) of Title 35, United States Code with regard to the invention entitled CANCER CHEMOPROTECTIVE FOOD PRODUCTS by inventor(s) FAHEY et al. described in

☒ the specification filed herewith  
☐ application serial no. \_\_\_\_\_, filed \_\_\_\_\_  
☐ patent no. \_\_\_\_\_, issued \_\_\_\_\_

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). \*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME: \_\_\_\_\_  
 ADDRESS: \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT CORPORATION

NAME: \_\_\_\_\_  
 ADDRESS: \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT CORPORATION

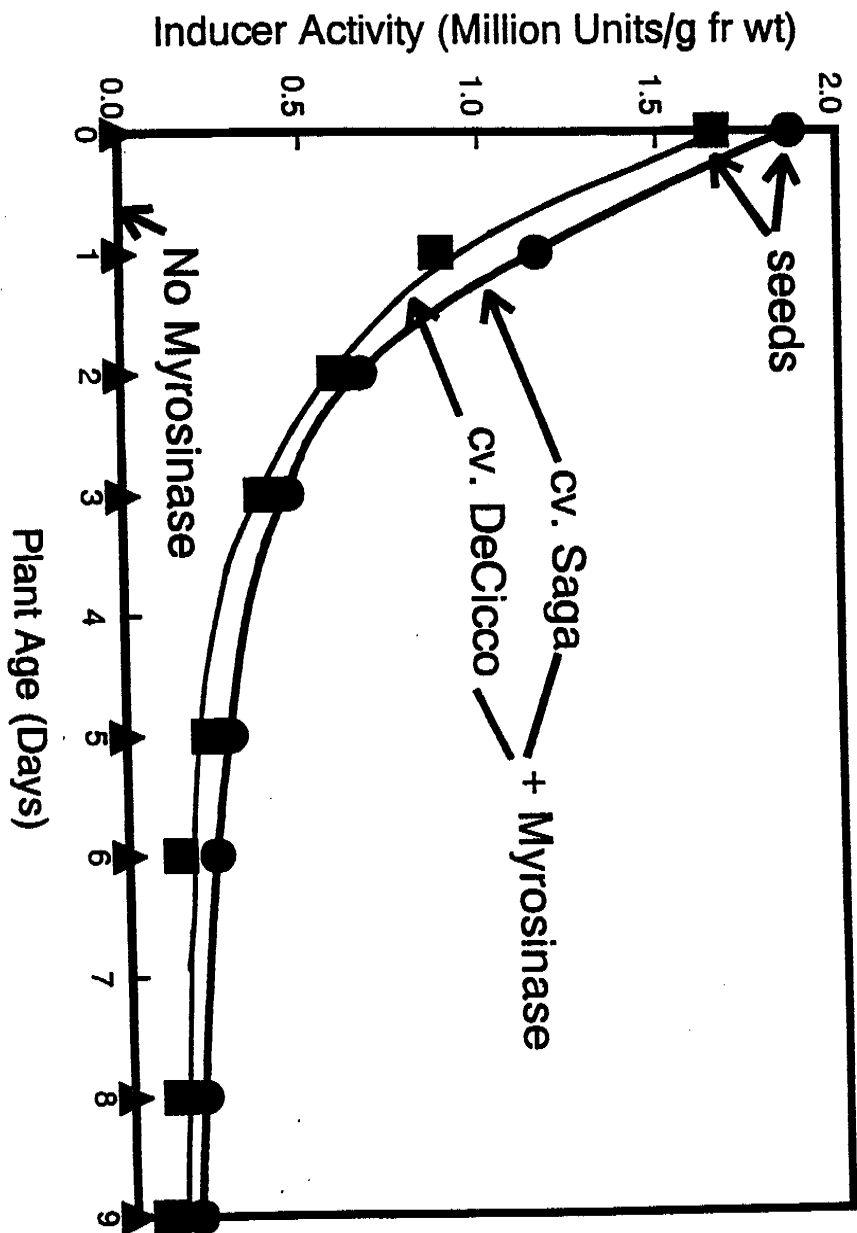
I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate: (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

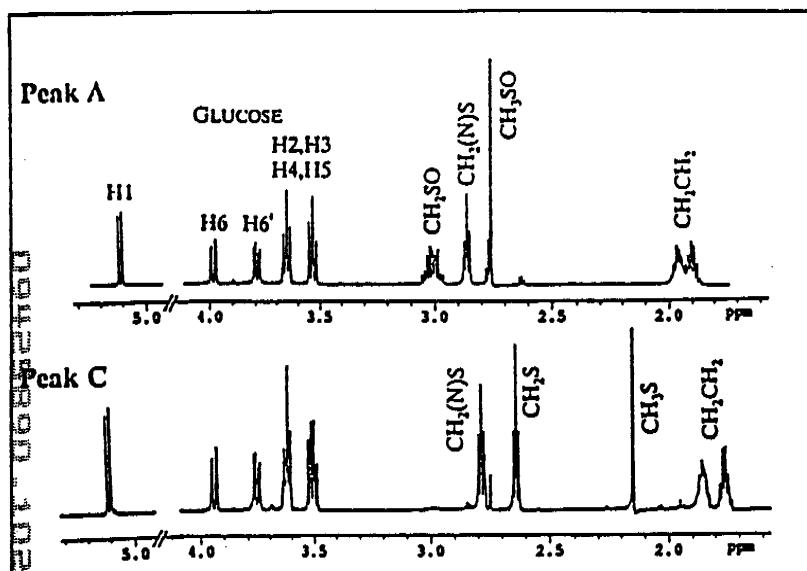
NAME OF PERSON SIGNING: David A. Blake, Ph.D.  
 TITLE OF PERSON OTHER THAN OWNER: Executive Vice Dean  
 ADDRESS OF PERSON SIGNING: 2720 Rutland Avenue, Baltimore, Maryland 21205  
 SIGNATURE: [Signature] DATE: 5/9/08

Figure 1

# Inducer Activity of Broccoli Sprouts Effect of Plant Age



▲ = < 1000 Units/g fr wt

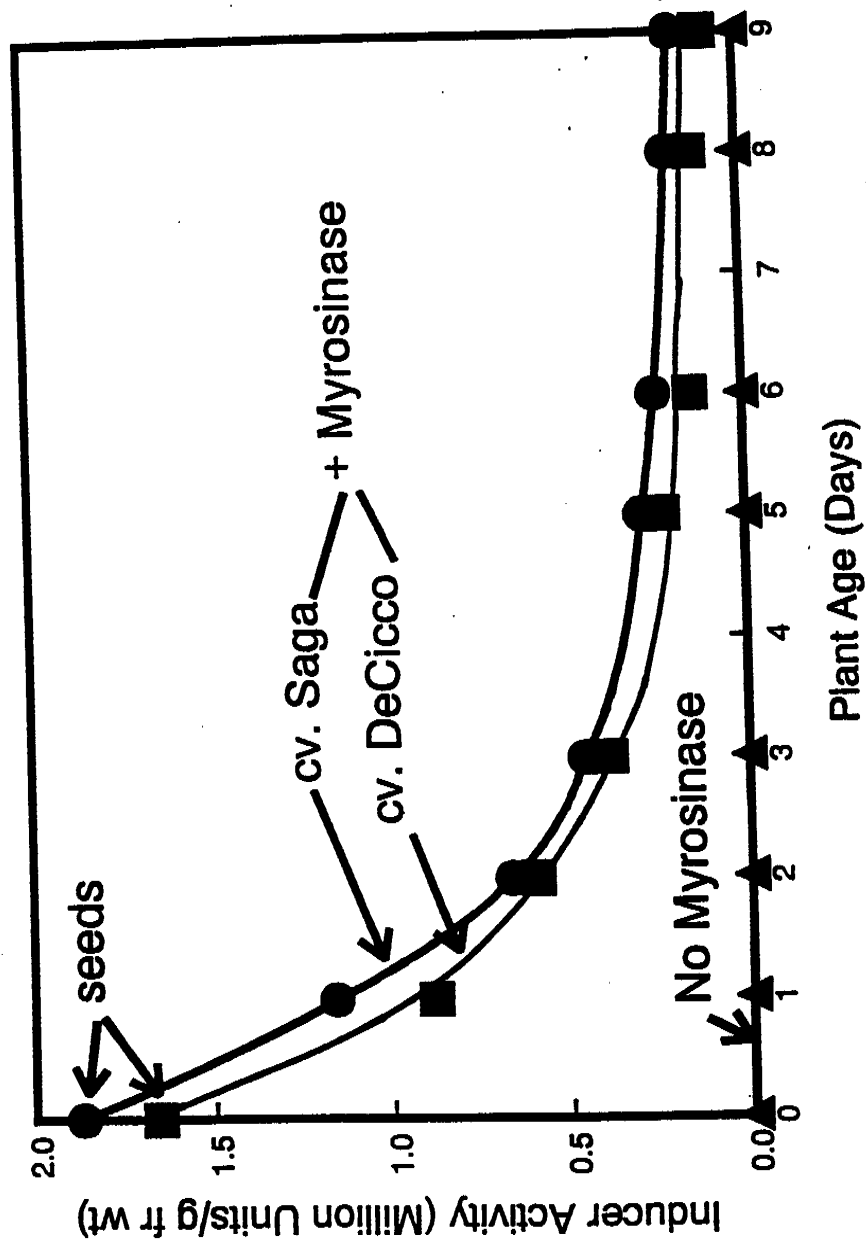


High Resolution NMR (600 MHz) in D<sub>2</sub>O. Note: chirality of SO in Peak A induces multiplet for CH<sub>2</sub>SO (Peak A), not observed for CH<sub>2</sub>S (Peak C).

Figure 2

# Inducer Activity of Broccoli Sprouts

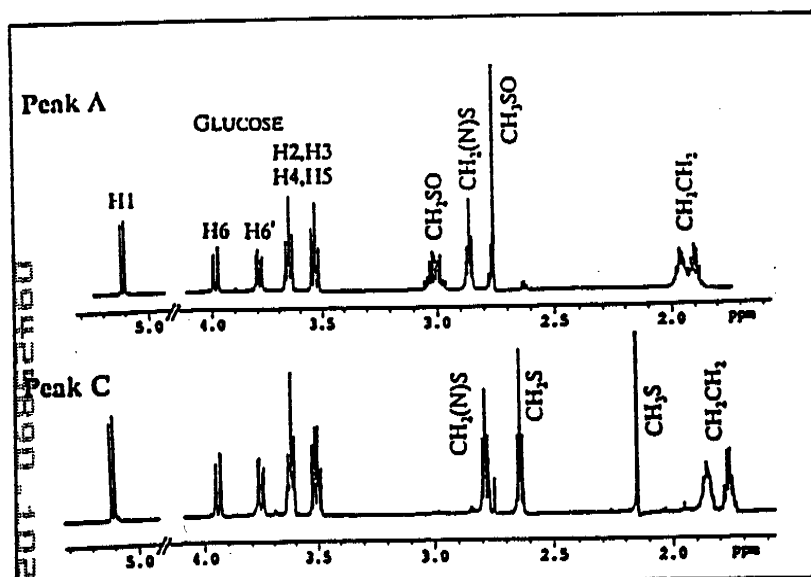
## Effect of Plant Age



▲ = < 1000 Units/g fr wt

Figure 1

PRINT OF DRAWINGS  
AS ORIGINALLY FILE



High Resolution NMR (600 MHz) in  $D_2O$ . Note: chirality of SO in Peak A induces multiplet for  $CH_2SO$  (Peak A), not observed for  $CH_2S$  (Peak C).

Figure 2

JHU-TECHNOLOGY LICENSING TEL: 410-955-1245

Apr 4.97 15:13 No.006 .02

ACE74 U.S. PTO  
09/425890  
10/25/99



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
ASSISTANT SECRETARY AND COMMISSIONER  
OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

MARCH 14, 1996

PTAS

B SAXE  
FOLEY & LARDNER  
P.O. BOX 25696  
3000 K STREET, N.W., SUITE 500  
WASHINGTON, D.C. 20007-5109



\*100091892A\*

MAR 20 1996

UNITED STATES PATENT AND TRADEMARK OFFICE  
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYER WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, NORTH TOWER BUILDING, SUITE 10C35, WASHINGTON, D.C. 20231.

RECORDATION DATE: 09/15/1995

REEL/FRAME: 7694/0746  
NUMBER OF PAGES: 2

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:  
FAHEY, JED W.

DOC DATE: 09/13/1995

ASSIGNOR:  
TALALAY, PAUL

DOC DATE: 09/13/1995

ASSIGNEE:  
JOHNS HOPKINS SCHOOL OF MEDICINE  
2024 E. MONUMENT STREET, SUITE 2-100  
BALTIMORE, MARYLAND 21205

SERIAL NUMBER: 08528858  
PATENT NUMBER:

FILING DATE: 09/15/1995  
ISSUE DATE:

SEDLEY PYNE, EXAMINER  
ASSIGNMENT DIVISION  
OFFICE OF PUBLIC RECORDS

JHU-TECHNOLOGY LICENSING TEL: 410-955-1245

Apr 4.97 15:13 No.006 P.

US PTO  
09/425890  
10/25/99

9-15-95 11-21-1995

FORM FID-1596 (modified)  
(Rev. 6-83)  
OMB No. 0651-0011 (exp. 4/98)

SEP 15 1995

U.S. DEPARTMENT OF COMMERCE  
Patent

11/15/95

To the Honorable Commissioner of Patents and Trademarks

1. Name of conveying party(ies): Jed W. FAHEY, Paul TALALAY

Additional name(s) of conveying party(ies) attached? No

2. Name and address of receiving party(ies):

Name: Johns Hopkins School of Medicine

Internal Address:

Street Address: 2024 E. Monument Street, Suite 2-100

City: Baltimore, State: MD ZIP: 21205

Additional name(s) & address(es) attached? No

3. Nature of conveyance:

☒ Assignment ☐ Merger  
☐ Security Agreement ☐ Change of Name  
☐ Other

Execution Date: 09-13-95

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is: 09-15-95

A. Patent Application No.(s)

B. Patent No.(s)

Additional numbers attached? No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: OLEY & LARDNER - Attn: B. Saxe

Internal Address: P.O. Box 25698

Street Address: 3000 K Street, N.W., Suite 500

City: Washington, D.C. ZIP: 20007-5109

6. Total number of applications and patents involved: 1

7. Total fee (37 C.F.R. § 3.41): \$40.00

☒ Enclosed

☐ Authorized to be charged to deposit account

8. Deposit account number:

(Attach duplicate copy of this page if paying by deposit account)

DO NOT USE THIS SPACE

110 MG 10/11/95 08528858 1 581 40.00 PK

9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Bernhard D. Saxe  
Name of Person Signing

[Signature]  
Signature

September 15, 1995  
Date

Total number of pages including cover sheet, attachments, and document: 2

Mail documents to be recorded with required cover sheet information to:  
Commissioner of Patents & Trademarks, Box Assignments  
Washington, D.C. 20231

Sent to Clerk 1-14-99



# ASSIGNMENT - WORLDV I

For good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, each undersigned inventor has sold and assigned, and by these presents hereby sells and assigns, unto

## JOHNS HOPKINS SCHOOL OF MEDICINE

its successors and assigns, the entire right, title and interest, so far as concerns the United States and the Territories and Possessions thereof and all foreign countries in and to the invention in

## CANCER CHEMOPROTECTIVE FOOD PRODUCTS

as set forth in his United States Patent Application

XX executed concurrently herewith  
 — executed on \_\_\_\_\_  
 — Serial No. \_\_\_\_\_ filed \_\_\_\_\_

said application for United States Letters Patent, including all divisional, renewal, substitute, continuation and Convention applications based in whole or in part upon said inventions or upon said applications, and any and all Letters Patent and reissues and extensions of Letters Patent granted for said inventions or upon said applications and every priority right that is or may be predicated upon or arise from said inventions, said applications, and said Letters Patent; said Assignee being hereby authorized to file patent applications in any or all countries on any or all said inventions in the name of the undersigned or in the name of said Assignee or otherwise as said Assignee may deem advisable, under the International Convention or otherwise; the Commissioner of Patents and Trademarks of the United States of America being hereby authorized to issue or transfer all said Letters Patent to said Assignee in accordance herewith; this assignment being under covenant, not only that full power to make the same is had by the undersigned, but also that such assigned right is not encumbered by any grant, license, or other right theretofore given, and that the undersigned will do all acts reasonably serving to ensure that the said inventions, patent applications and Letters Patent shall be held and enjoyed by said Assignee as fully and entirely as the same could have been held and enjoyed by the undersigned if this assignment had not been made, and particularly to execute and deliver to said Assignee all lawful documents including petitions, specifications, oaths, assignments, invention disclaimers, and lawful affidavits in form and substance which may be requested by said Assignee, to furnish said Assignee with all facts relating to said inventions or the history thereof and any and all documents, photographs, models, samples or other physical exhibits which may be of said inventions, and to testify in any proceedings relating to said inventions, patent applications and Letters Patent.

The undersigned hereby grant the firm of FOLEY & LARDNER the power to insert in this Assignment any further identification which may be necessary or desirable to comply with the rules of the U.S. Patent and Trademark Office for recordation of this Assignment.

NAMES AND SIGNATURES OF INVENTORS		
Name: JED W. FAHEY	Signature: <i>Jed W. Fahey</i>	Date: 9/13/95
Name: PAUL TALALAY	Signature: <i>Paul Talalay</i>	Date: 9/13/95
Name:	Signature:	Date:
NAMES AND SIGNATURES OF WITNESSES		
Name: RUTH DILLINGER	Signature: <i>Ruth Dillinger</i>	Date: 9/13/95
Name: SHARON KERRY	Signature: <i>S. Kerry</i>	Date: 9.13.95

Note: Prima facie evidence of execution may optionally be obtained by execution of this document before a U.S. Consul or before a local officer authorized to administer oaths whose authority is proved by a certificate from a U.S. Consul.

2

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Paper Number

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 046528/0121

2/A  
C.F.  
2/3/06

In re patent application of

Jed FAHEY *et al.*

Serial No. Unassigned

Filed: October 25, 1999

For: CANCER CHEMOPROTECTIVE FOOD PRODUCTS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, Applicants respectfully request that the following amendments be entered into the application:

IN THE CLAIMS:

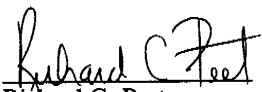
Kindly cancel claims 1-35 and 39-47 without prejudice or disclaimer.

REMARKS

Claims 36-38 are now pending. Claims 1-35 and 39-47 have been canceled. Entry of the foregoing amendment prior to examination is respectfully requested.

Respectfully submitted,

October 25, 1999

  
Richard C. Peet  
Registration No. 35,792

FOLEY & LARDNER  
3000 K Street, N.W.  
Suite 500  
Washington, D.C. 20007-5109  
Tel: (202) 672-5300

046528/0121



Law Offices  
FOLEY & LARDNER  
Suite 500  
3000 K Street, N.W.  
Washington, DC 20007-5109  
(202) 672-5300

*Pre-Amend  
A*



TO: Assistant Commissioner for Patents  
Box Patent Applications  
Washington D.C. 20231

Attorney Docket No.046585/0121

**UTILITY PATENT APPLICATION TRANSMITTAL**  
**(new nonprovisional applications under 37 CFR 1.53(b))**

Transmitted herewith for filing is the patent application of:

INVENTOR(S): Jed W. FAHEY and Paul TALALAY

TITLE: CANCER CHEMOPROTECTIVE FOOD PRODUCTS

In connection with this application, the following are enclosed:

**APPLICATION ELEMENTS:**

XX Specification - 51 TOTAL PAGES

(preferred arrangement:)

- Descriptive Title of the Invention
- Cross Reference to Related Applications
- Statement Regard Fed sponsored R&D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

XX Drawings - Total Sheets 2

XX Declaration and Power of Attorney - Total Sheets 2

\_\_\_ Newly executed (original or copy)

XX Copy from a prior application (37 CFR 1.63(d))

(relates to continuation/divisional boxes completed) - NOTE: Box below

\_\_\_ DELETION OF INVENTOR(S) - Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).

XX Incorporation By Reference (useable if copy of prior application Declaration being submitted)

The entire disclosure of the prior application, from which a COPY of the oath or declaration is supplied as noted above, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

\_\_\_ Microfiche Computer Program (Appendix)

\_\_\_ Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)

\_\_\_ Computer Readable Copy

\_\_\_ Paper Copy (identical to computer copy)

\_\_\_ Statement verifying identify of above copies

**ACCOMPANYING APPLICATION PARTS**

\_\_\_ Assignment Papers (cover sheet & document(s))

\_\_\_ 37 CFR 3.73(b) Statement (when there is an assignee)

\_\_\_ English Translation Document (if applicable)

\_\_\_ Information Disclosure Statement(IDS) with PTO-1449. \_\_\_ Copies of IDS Citations

XX Preliminary Amendment

XX Return Receipt Postcard (MPEP 503)

Utility Patent Application Transmittal  
 Attorney Docket No. 046 /0118 - Foley & Lardner  
 Page 2

☒ XX Small Entity Statement(s)  
☒ XX Statement filed in prior application, status still proper and desired.  
☐ — Certified Copy of Priority Document(s) with Claim of Priority  
       (if foreign priority is claimed).  
☐ — OTHER:

If a **CONTINUING APPLICATION**, check appropriate box and supply the requisite information:

☐ — Continuation ☒ XX Divisional ☐ — Continuation-in-part (CIP)  
       of prior application Serial No. 09/118,867, filed July 20, 1998,  
pending; which is a divisional of 08/840,234, filed April 11, 1997, now  
U.S. Patent 5,968,567, issued 10-19-99.

XX Amend the specification by inserting before the first line the following sentence: ---This application is a divisional of prior application Serial No. 09/118,867, filed July 20, 1998, now pending; which is a divisional of 08/840,234, filed April 11, 1997, now U.S. Patent 5,968,567, issued 10-19-99.---

**CORRESPONDENCE ADDRESS:**

Foley & Lardner Address noted above.  
 Telephone: 202-672-5300  
 Fax Number: 202-672-5399

**FEE CALCULATIONS:** (Small entity fees indicated in parentheses.)

(1) For	(2) Number Filed	(3) Number Extra	(4) Rate	(5) Basic Fee \$760 (\$380)
Total Claims	3 - 20 =	0	x \$18 (x \$9)	0
Independent Claims	2 - 3 =	0	x \$78 (x \$39)	0
Multiple Dependent Claims			\$260 (\$130)	0
Assignment Recording Fee per property			\$40	0
Surcharge Under 37 C.F.R. 1.16(e)			\$130 (\$65)	0
TOTAL FEE:				\$380.00

**METHOD OF PAYMENT:**

A check in the amount of the above TOTAL FEE is attached. If payment by check is NOT enclosed, it is requested that the Patent and Trademark Office advise the undersigned of the period of time within which to file the TOTAL FEE. If payment enclosed, this amount is believed to be correct; however, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 19-0741.

Respectfully submitted,

Date: October 25, 1999  
 Docket No.: 046585/0121

Richard C. Peet  
 Richard C. Peet  
 Reg. No. 35,792

3

3

Paper Number

3

3

3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of  
Jed FAHEY et al.

Serial No. 09/425,890

Filed: October 25, 1999

For: CANCER CHEMOPROTECTIVE FOOD PRODUCTS



Attorney Docket No. 046585/0121

Group Art Unit: 1761

Examiner: Unknown

**INFORMATION DISCLOSURE STATEMENT**  
**UNDER 37 C.F.R. §1.56**

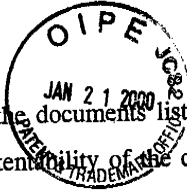
Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Submitted herewith on a modified Form PTO-1449 is a listing of documents known to applicants in order to comply with applicant's duty of disclosure pursuant to 37 C.F.R. §1.56.

The documents listed are cited in the parent application. The listed documents include documents that became known to applicant incident to a suit for infringement of U.S. Patent No. 5,725,895 filed in the District Court of Delaware. U.S. Patent No. 5,725,895 is not related to the present application. However, applicants note that the present application and U.S. Patent No. 5,725,895 have an inventor in common and relate, generally, to the same technical field. Accordingly, out of an abundance of caution, and in compliance with the duty of disclosure, applicant hereby brings these documents to the attention of the Patent Office

The accompanying Form PTO-1449 lists several papers and publications that were provided during the course of discovery in the infringement suit. In addition, the defendants have recently filed a request for reexamination of U.S. Patent No. 5,725,895 citing several of the listed papers and publications.



Applicant believes that the documents listed in the accompanying Form PTO-1449 do not adversely impact the patentability of the claims of the above-captioned application. However, out of an abundance of caution, and in compliance with the duty of disclosure, applicant hereby brings these documents to the attention of the Patent Office.

In the course of the infringement suit related to U.S. Patent No. 5,725,895, the defendants also have lodged several affirmative defenses and counterclaims, including (1) invalidity and unenforceability for failure to comply with the provisions of 35 U.S.C. §§ 101, 102, 103, and 112, (2) breach of the duty to disclose material information, and (3) patent misuse. The defendants' "Answer, Affirmative Defenses and Counterclaim," which contains these allegations, also is listed on the accompanying Form 1449.

Applicant believes that the foregoing affirmative defenses and counterclaims are without merit. However, out of an abundance of caution, and in compliance with the duty of disclosure, applicant hereby brings these documents to the attention of the Patent Office.

The submission of any document herewith, which is not a statutory bar, is not intended as an admission that such document constitutes prior art against the claims of the present application or that such document is considered material to patentability as defined in 37 C.F.R. §1.56(b). Applicant does not waive any rights to take any action which would be appropriate to antedate or otherwise remove as a competent reference any document which is determined to be a *prima facie* prior art reference against the claims of the present application.

**CONCISE EXPLANATION OF  
RELEVANCE OF EACH DOCUMENT**

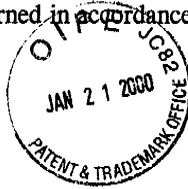
Applicants are submitting herewith on Form PTO-1449, a listing of the documents cited by or submitted to the Patent Office in parent application Serial No. 09/118,867, filed July 20, 1998, which is a divisional application of Serial No. 08/840,234, filed April 11, 1997, now U.S. Patent No. 5,968,567. The relevance of these prior art documents is explained in the parent application.



As provided in 37 C.F.R. §1.98(d), copies of the documents are not being provided since they were previously cited by or submitted to the Patent Office in parent application Serial No. 08/840,234, filed April 11, 1997, which is a divisional application of Serial No. 08/528,858, filed September 15, 1995, now U.S. Patent No. 5,725,895.

Since this Information Disclosure Statement is being filed in compliance with 37 C.F.R. §1.97(b) before mailing of a first Office Action on the merits, no fee is required in connection with its filing.

Applicant respectfully requests that the listed documents be considered by the Examiner and be made of record in the present application and that an initialed copy of Form PTO-1449 be returned in accordance with MPEP §609.



Respectfully submitted,

JANUARY 21, 2000  
Date

Richard C. Peet  
for Richard C. Peet  
Registration No. 35,792

Reg No 40,960

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PATENT & TRADEMARK OFFICE

Form PTO-1449 (MODIFIED)	U.S. DEPT. OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET 046585/0121	SERIAL NO. 09/425,890
<b>2 INFORMATION DISCLOSURE CITATION</b> Date Submitted to PTO: January 20, 2000		APPLICANT Jed W. FAHEY et al.	
		FILING DATE October 25, 1999	GROUP ART UNIT 1761

Sheet 1 of 4

U.S. PATENT DOCUMENTS							
EXAMINER INITIAL	REF	DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
IAW	A1	5,725,895	3/1998	Fahey et al.	426	49	
IAW	A2	5,411,986	5/1995	Cho et al.	514	514	
	A3						
	A4						
	A5						
	A6						
	A7						

FOREIGN PATENT DOCUMENTS								
	REF	DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	TRANSLATION	
							YES	NO
	A8							
	A9							
	A10							
	A11							

OTHER DOCUMENT(S) (Including Author, Title, Date, Pertinent Pages, Etc.)			
IAW	A12		The Sproutletter, Number 25, Nov. - Dec. 1984.
	A13		"The Sproutletter" May-June 1981, No. 4.
	A14		Roy Bruder, Ph.D., Discovering Natural Foods, (including pgs.203-209), Woodbridge Press, 1982.
	A15		Brian R. Clement, Hippocrates Health Program, (including pgs 7-11), Hippocrates Publications, 1989.
	A16		Jethro Kloss, The Back to Eden Cookbook, pgs. 61-61, Woodbridge Press, 1974.
	A17		Steve Meyerowitz, Sproutmann Kitchen Garden Cookbook, The Sprouthouse, Inc., pgs. 178-179, 290, 1994.
	A18		Steve Meyerowitz, Sprout It, One week from Seed to Salad, The Sprouthouse, Inc., (including pgs. 84-85, 120-123), June 1994.
	A19		Steve Meyerowitz, The Complete Guide to Sprouting, Sprouts The Miracle Food, Sproutman Publications, (including pgs. 121-2), May 1998.
	A20		Esther Munroe, Sprouts to Grow and Eat, (including pgs. 2-15), Dec. 1974.
	A21		Jean Hewitt, The New York Times "New Natural Foods Cookbook", Avon Books, pgs. 200-203, 1982.
	A22		Martha H. Oliver, Add a Few Sprouts To Eat Better for Less Money, Pivot Original Health Books, (including pgs. 52-53, 118-119), 1975.
	A23		James C. Schmidt, Horticulture Facts, "Growing Sprouts Indoors", (Rev. 4/81).
IAW	A24		Angnes Toms, The Joy of Eating Natural Foods, The Complete Organic Cookbook, pgs. 318-319, Nov. 1971.

Leslie Wong

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B INFORMATION DISCLOSURE CITATION Date Submitted to PTO: January 20, 2000		APPLICANT Jed W. FAHEY et al.	
		FILING DATE October 25, 1999	GROUP ART UNIT 1761

Sheet 2 of 4

LAW	A25	Karen Cross Whyte, The Complete Sprouting Cookbook, Troubador Press, (including pgs. 57-59), 1973.
	A26	Ann Wigmore, The Sprouting Book, Avery Publications, (including pgs. 29-37), 1986.
	A27	Debra Schwarze, Growing Sprouts, Neb Guide, Jan. 1989.
	A28	John Tobe, Sprouts Elixir of Life", 1970.
	A29	Alicia Bay Laurel, "Living on the Earth" a Vintage Book, date N.A.
	A30	David Ehrlich with George Wolf, Foreward by Peter Albright, M.D., "The Bowell Book", Schocken Books, 1981.
	A31	"The Good News Sprouts Recipe Book" ISGA, Aug. 1992.
	A32	Ann Wigmore, "The Hippocrates Diet and Health Program", Avery Publications, 1984.
	A33	Sprouting Publications Oahspe Foundation, Health and Sprouting Supplies, date N.A.
	A34	Sproutletter, #41, Summer, 1989.
	A35	The Sproutletter, Number 27, March-April 1985.
	A36	Steve Meyerowitz, Growing Vegetables Indoors", 1983.
	A37	The Sproutletter, Number 24, Sept.-Oct. 1984.
	A38	The Sproutletter, Issue 33, Spring 1987.
	A39	The Sproutletter, Number 28, May-June 1985.
	A40	The Sproutletter, Number 26, Jan-Feb 1985.
	A41	Sprouting Publications, Health and Sprouting Supplies, date N.A.
	A42	The Sproutletter, Number 29, July - August 1985.
	A43	Sproutletter, #40, Spring, 1989.
	A44	The Sproutletter, Number 32, Summer, date N.A.
	A45	Sproutletter, #44, March 1991.
	A46	Sproutletter, #36, Winter, 1987-88.
	A47	Sproutletter, #39, Fall, 1988.
	A48	Sproutletter, #43, May/June 1990.
	A49	Sproutletter, #38, Summer, 1988.
LAW	A50	Sprouting Publications Health and Sprouting Supplies, date N.A.
	A51	Spring Sale for Members Only.
LAW	A52	The Sproutletter, A newsletter of useful and unusual information on sprouts, raw foods and nutrition, date N.A.
	A53	The Sproutletter, #31, Winter, date N.A.
	A54	Deirdre Purdy, ed., The Summer Kitchen, A Farmers' Market Cookbook, 1981.
	A55	Viktoras Kulvinskis, M.S. Co-Director Hippocrates Health Institute, "Love Your Body or how to be a live food lover", 1974.
	A56	The Sprout House Article from Newspaper, date N.A.
	A57	New Prices - New Products, july 1985 order form.
	A58	Steve Meyerowitz, Indoor Vegetable Kit, The Sprout House, date N.A.
LAW	A59	The Sprout House Newsletter, Issue #15, August, 1992.

Leslie Wong

8/8/00

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Form PTO-1449 (MODIFIED)	U.S. DEPT. OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET 046585/0121	SERIAL NO. 09/425,890
INFORMATION DISCLOSURE CHART		APPLICANT Jed W. FAHEY et al.	
Date Submitted to PTO: January 20, 2000		FILING DATE October 25, 1999	GROUP ART UNIT 1761



Sheet 3 of 4

	A60	Sproutman's Organic Seeds for Sprouting 100% Organically Grown Order Form.
	A61	Complaint for Patent Infringement (Brassica Protection Products, LLC v. The Sproutman, Inc. dated September 20, 1999.
	A62	Murry Tizer's Answer, Affirmative Defenses and Counterclaims dated June 28, 1999.
	A63	The Sproutman, Inc.'s Answer, Affirmative Defenses and Counterclaims dated June 28, 1999.
	A64	Request for Reexamination of U.S. Patent No. 5,725,895 filed October 11, 1999.
AW	A65	Sprout it! One Week From Seed to Salad, Steve Meyerowitz (The Sprout House, Inc., Great Barrington, MA),. Pages 20-21, 58, 85-86, 120-123, 1983.
	A66	Munroe, E., Sprouts to Grow and Eat, the Steven Greene Press, (1974), pp. 2-9 and 14-15.
	A67	Schmidt, James C., Growing Sprouts Indoors, Cooperative Extension Service of the University of Illinois at Urbana-Champaign, College of Agriculture (1984) (pamphlet).
	A68	Whyta, K.C., The Complete Sprouting Cookbook, Troubador Press (1983), pp 10-16, 57-60.
	A69	The Sprout House cookbook, Steve Meyerowitz (The Sprout House, Inc. Great Barrington, MA 1983), pages 20-21, 85, 120-123.
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	A71	Posner et al., "Design and Synthesis of Bifunctional Isothiocyanate Analogs of Sulforaphane Correlation between structure and potency as Inducers of Anticarcinogenic Detoxication Enzymes", Journal of Medicinal Chemistry, Vol. 37, No. 1, pp. 170-175, 1994.
	A72	Zhang et al., "A major inducer of Anticarcinogenic protective enzymes from broccoli: isolation And education of structure", Proc. Natl. Acad. Sci. USA, Vol. 89, pp. 2399-2403, March 1992.
	A73	Prochaska et al. "Rapid detection of inducers of enzymes that protect against carcinogens", Proc. Nat'l. Sci. USA Vol. 89, pp. 2394-2388, March 1992.
	A74	Zhang et al., "Anticarcinogenic activities of sulforaphane and structurally related synthetic norbomyl isothiocyanates", Proc. Natl. Sci. USA, Vol. 91, pp. 3147-3150, April 1994.
	A75	Prochaska et al., "Regulatory Mechanisms of Monofunctional and Bifunctional Anticarcinogenic Enzyme Inducers in Murine Liver," Cancer Research Vol. 48, pp. 4776-4782, September 1988.
	A76	Prochaska et al., "Direct Measurement of NAD(P)H: Quinone Reductase from Cells Cultured in Microtiter Wells: A Screening Assay for Anticarcinogenic Enzyme Inducers", analytical Biochemistry, vol. 169, pp. 328-336, 1988.
	A77	Beecher, "Cancer prevention properties of varieties of Brassica oleracea: a review 1-3", Am J. Clin. Nutr.: 59 (suppl.) pp. 1166 s-1170s, 1994.
AW	A78	Presters et al., "Chemical and molecular regulation of enzymes that detoxify carcinogens", Proc. Natl. Acad. Sci. USA, Vol. 90, pp. 2965-2969, April 1993.

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4-C44

Form PTO-1449	U.S. DEPT. OF COMMERCE	ATTY. DOCKET	SERIAL NO.
(MODIFIED)	PATENT AND TRADEMARK OFFICE	046585/0121	09/425,890
<b>INFORMATION DISCLOSURE CITATION</b>  <i>Date Submitted to PTO: January 20, 2000</i>		APPLICANT	Jed W. FAHEY et al.
		FILING DATE	GROUP ART UNIT
		October 25, 1999	1761

Sheet 4 of 4

EXAMINER		DATE CONSIDERED
<div style="text-align: center;">  </div>	A79	Zhand et al., "Anticarcinogenic Activities of Organic Isothiocyanates: Chemistry and Mechanisms", Cancer Research suppl., 54, pp. 1976s-1981s, April 1, 1994.
	A80	Talalay, "The role of Enzyme Induction in Protection Against Carcinogenesis", Cancer Chemoprevention, pp. 469-478, 1992.
	A81	Prestera et al., "The Electrophile Counterattack Response: Protection Against Neoplasia and Toxicity", Advan. Enzyme Regul., vol. 33, pp. 281-296, 1993.
	A82	Masilungan et al., "Screening of Philippine Medicinal Plants for Anticancer Agents using CCNSC "Protocols", Cancer Chemotherapy Reports (Part 2) Vol. 2, No.1, pp. 135-140, April 1971.
	A83	Polasa et al., "Cancer preventive properties of varieties of Brassica oleracea: A review Source", American Journal of Clinical Nutrition 59 (5 Suppl), 1994.
<div style="text-align: center;">  </div>	A84	Patent Abstract of Japan Sect. No. 305, Vol. 9, No. 2371, p.2, September 1985.
	A85	Barrett et al., "Protective Effect of Cruciferous Seed Meals Against Mouse Colon Cancer", Cereal Foods World 613, Vol. 39, No. 8, pp. 613, August 1994.

\* EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include any copy of this form with next communication to applicant.

4

4

Paper Number

4

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Attorney Docket No. 046585/0121

In re patent application of:

Jed FAHEY *et al.*

Serial No.: 09/425,890

Filed: October 25, 1999

FOR: CANCER CHEMOPROTECTIVE FOOD PRODUCTS



Group: Unassigned

Examiner: Unassigned

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, DC 20231

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TC 1700 MAIL ROOM

Sir:

Prior to examination of the above-identified application, Applicants respectfully request that the following amendments be entered into the application:

**IN THE CLAIMS:**

Please add the following new claims:

- sub c21*
- ~~18.~~ A method of preparing a human food product comprising cruciferous seeds, flour made from the cruciferous seeds, or a combination thereof, wherein the cruciferous seeds or flour contain high Phase 2 enzyme-inducing potential, comprising the steps of:
- B1*
- (a) selecting cruciferous seeds which produce sprouts that contain at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth, and
  - (b) preparing a food product from the selected cruciferous seeds.

Serial No. Unassigned

Attorney Docket No.: 046585/121

<sup>8</sup>  
49. The method of claim <sup>7</sup>48, wherein the selected cruciferous seeds produce sprouts that contain at least 300,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth.

<sup>9</sup>  
50. The method of claim <sup>7</sup>48, wherein the selected cruciferous seeds produce sprouts that contain at least 400,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth.

B1  
cont

<sup>10</sup>  
51. The method of claim <sup>7</sup>48, wherein the selected cruciferous seeds produce sprouts that contain at least 500,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth.--

#### REMARKS

The foregoing amendments add new claims 48-51 to the present application. Applicants respectfully request that these amendments be made to the present application prior to examination.



Serial No. Unassigned

Attorney Docket No.: 046585/121

Upon entry of the foregoing amendments, claims 36-38 and 48-51 are presented for examination. Claims 1-35 and 39-47 are canceled without prejudice or disclaimer.

Respectfully submitted,

November 30, 1999  
Date

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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees.